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Serial Review: Flavonoids and Isoflavones (Phytoestrogens): Absorption, Metabolism, and Bioactivity
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POTENTIAL TOXICITY OF FLAVONOIDS AND OTHER DIETARY PHENOLICS: SIGNIFICANCE FOR THEIR CHEMOPREVENTIVE AND ANTICANCER PROPERTIES

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Abstract—Flavonoids, including isoflavones, are natural components in our diet and, with the burgeoning interest in alternative medicine, are increasingly being ingested by the general population. Plant phenolics, which form moieties on flavonoid rings, such as gallic acid, are also widely consumed. Several beneficial properties have been attributed to these dietary compounds, including antioxidant, anti-inflammatory, and anticarcinogenic effects. Flavonoid preparations are marketed as herbal medicines or dietary supplements for a variety of alleged nontoxic therapeutic effects. However, they have yet to pass controlled clinical trials for efficacy, and their potential for toxicity is an understudied field of research. This review summarizes the current knowledge regarding potential dietary flavonoid/phenolic-induced toxicity concerns, including their pro-oxidant activity, mitochondrial toxicity (potential apoptosis-inducing properties), and interactions with drug-metabolizing enzymes. Their chemopreventive activity in animal in vivo experiments may result from their ability to inhibit phase I and induce phase II carcinogen metabolizing enzymes that initiate carcinogenesis. They also inhibit the promotion stage of carcinogenesis by inhibiting oxygen radical-forming enzymes or enzymes that contribute to DNA synthesis or act as ATP mimics and inhibit protein kinases that contribute to proliferative signal transduction. Finally, they may prevent tumor development by inducing tumor cell apoptosis by inhibiting DNA topoisomerase II and p53 downregulation or by causing mitochondrial toxicity, which initiates mitochondrial apoptosis. While most flavonoids/phenolics are considered safe, flavonoid/phenolic therapy or chemopreventive use needs to be assessed as there have been reports of toxic flavonoid–drug interactions, liver failure, contact dermatitis, hemolytic anemia, and estrogenic-related concerns such as male reproductive health and breast cancer associated with dietary flavonoid/phenolic consumption or exposures.

Keywords—Flavonoids, Isoflavones, Chemoprevention, Anticancer, Toxicity, Free radicals

INTRODUCTION

Cancer is the second leading cause of death in the United States and in many other nations in the world. The prognosis for a patient with metastatic carcinoma of the lung, colon, breast, or prostate remains a concern and accounts for more than half of all cancer deaths. Chemoprevention or chemotherapy via nontoxic agents could be one approach for decreasing the incidence of these cancers. Many naturally occurring agents have shown chemopreventive and chemotherapeutic (anticancer) potential in a variety of bioassay systems and animal models. An effective and acceptable chemopreventive or anticancer agent should have certain properties: (i) no toxic effects in normal and healthy cells, (ii) high efficacy against multiple cancers, (iii) capability of oral consumption, (iv) known mechanism of action, (v) low

This article is part of a series of reviews on "Flavonoids and Isoflavones (Phytoestrogens): Absorption, Metabolism, and Bioactivity." The full list of papers may be found on the home page of the journal.

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cost, and (vi) acceptance by the human population [1]. In order to suggest that dietary flavonoids may be such agents, a critical review of their potential toxic effects is necessary and is provided here.

Flavonoids are constituents of fruits, vegetables, nuts, plant-derived beverages such as tea and wine, and traditional Eastern medicines such as *Ginkgo biloba*, as well as components present in a plethora of herbal-containing dietary supplements. Humans have consumed flavonoids and other dietary phenolics since the arrival of human life on earth. Over 4000 different naturally occurring flavonoids have been described [2] and the list is still growing. Flavonoids have been known as plant pigments for over a century and belong to a vast group of phenolic compounds that are widely distributed in all foods of plant origin. In the normal North American diet, flavonoid glycosides are unavoidably consumed daily, with an estimated total consumption of 1 g/d [3], which could be much higher if dietary supplements are also consumed. As an example, dietary supplements of quercetin have been suggested to contain doses which are up to 20 times higher than those which would be obtained in a typical vegetarian diet [4]. Recent work is beginning to highlight the potential health-beneficial properties of flavonoids, known to be powerful antioxidants in vitro [5,6]. Compelling data from in vitro and in vivo laboratory studies, epidemiological investigations, and human clinical trials indicate that flavonoids have important effects on cancer chemoprevention and therapy. However, the potential toxicity of these dietary components has not been well studied.

Flavonoids may interfere in several of the steps that lead to the development of malignant tumors, including protecting DNA from oxidative damage, inhibiting carcinogen activation, and activating carcinogen-detoxifying systems, reviewed by us and others [7–9]. This review will focus on the various toxicities, chemopreventive, and anticancer properties associated with flavonoids and other dietary phenolics. Their pro-oxidant activity and ability to induce mitochondrial dysfunction will be discussed as possible anticancer mechanisms, whereby these effects may lead to apoptosis of tumor cells. Various chemopreventive properties, such as the inhibition of enzymes responsible for carcinogen activation and therefore prevention of tumor formation, will also be reviewed. These chemopreventive properties may lead to toxicity as the inhibition of carcinogen-activating enzymes may also cause potential toxic flavonoid–drug interactions. In addition, dietary phenolics with similar structures and similar pharmacological effects, or phenolics which form the moieties of certain flavonoids, will also be discussed, as the recent literature regarding these dietary phenolics will complement that of flavonoids and make for a more complete discussion. This knowledge will be beneficial when assessing potential flavonoid toxicity and chemopreventive or anticancer properties.

POTENTIAL DIETARY FLAVONOID/PHENOLIC TOXICITY

Pro-oxidant activity in the presence of transition metals or peroxidases

Dietary phenolics have been shown to act as pro-oxidants in systems containing redox-active metals. In the presence of O_2, transition metals such as copper (Cu) and iron (Fe) catalyze the redox cycling of phenolics, leading to the formation of reactive oxygen species (ROS) and phenoxyl radicals that can damage DNA, lipids, and other biological molecules [10–12]. Exposure of DNA to dihydrocaffeic acid in the presence of Cu resulted in more DNA single- and double-strand breaks than caffeic acid, whereas chlorogenic acid caused only minimal damage even though these phenolics had similar structures and redox potential. The authors proposed that the initial oxidation of the catechols by Cu^{2+} generated a semiquinone that reacted with O_2 to form $O_2^{\bullet -}$, which then oxidized the catechol to regenerate the semiquinone and H_2O_2. H_2O_2 was then rapidly converted by Cu^{1+} to the $^{\bullet}OH$ radical in a Fenton-type reaction [13].

Flavonols with pyrogallol or catechol B rings have also been shown to autoxidize in the presence of transition metals to produce ROS which accelerate low-density lipoprotein oxidation during the propagation phase [14]. However, in vivo, most transition metal ions are sequestered in forms unable to catalyze free radical reactions [15]. Very low levels of free copper ions may be released by tissue injury (e.g., atherosclerotic lesions) [16] and possibly hepatic Cu(II) overload diseases such as Wilson disease. The green tea catechin, epigallocatechin gallate (EGCG), was recently shown to induce H_2O_2 generation and cause subsequent oxidative damage to isolated and cellular DNA in the presence of transition metal ions [17]. EGCG also significantly induced DNA oxidation in HL-60 cells likely due to intracellular myeloperoxidase. DNA oxidation induced by EGCG was further increased in glutathione (GSH) depleted cells. However, this did not occur in H_2O_2-resistant HP100 cells, which are reported to have catalase activity which is 18 times higher than that of HL-60 cells [17], suggesting a role for H_2O_2 generation by EGCG involved in DNA oxidation. This pro-oxidant activity could explain why EGCG enhanced dimethylhydrazine or nitrosamine colon carcinogenesis in rats [18].

A particular focus in our laboratory is peroxidase-catalyzed oxidation of phenol ring-containing flavonoids and other dietary phenolics to phenoxyl radicals.

Peroxidases are heme-containing enzymes that usually catalyze a one-electron oxidation of a variety of xenobiotics by hydrogen peroxide [19]. Myeloperoxidase, eosinophil peroxidase, and lactoperoxidase are primarily found in granules (lysosomes) of neutrophils, eosinophils, and secretory cells of the exocrine gland, respectively. Myeloperoxidase and eosinophil peroxidase are released into the phagocytic vacuole and the plasma, whereas lactoperoxidase is secreted into milk, saliva, and tears [20].

Xenobiotics or their metabolites accumulating in the plasma or bone marrow may also be co-oxidized by activated leukocytes. Secondary acute myelogenous leukemias occurring after cancer therapy with etoposide (a phenolic topoisomerase inhibitor) or leukemia from chronic exposure to benzene (phenol metabolite) has been attributed to DNA damage by pro-oxidant phenoxyl radicals formed by myeloperoxidase/H_2O_2 [21–23]. Redox cycling of phenoxyl radicals co-oxidized GSH to form thiyl radicals and ROS [24].

HL-60 cells are a bone marrow-derived leukemia cell line and they contain myeloperoxidase. Incubation of HL-60 cells with phenol or etoposide and H_2O_2 caused cellular GSH to be oxidized to thiyl radicals, which was prevented by myeloperoxidase inhibitors. Etoposide phenoxyl radical formation was detected only in GSH-depleted HL-60 cells and was prevented by myeloperoxidase inhibitors or competitive antioxidant substrates [21]. In addition, DNA deoxyguanosine, ascorbate, and phospholipids were oxidized, presumably by the phenoxyl radicals initially formed [22].

Phenoxyl radicals have also been shown to be involved in the initiation stage of atherosclerosis as myeloperoxidase/H_2O_2 readily catalyzed the oxidation of tyrosine, a plasma phenol, to a tyrosyl radical which then co-oxidized low density lipoprotein (LDL) [25]. Activated neutrophils catalyzed the oxidation of tyrosine [26]. The tyrosyl phenoxyl intermediates also caused radical lipid peroxidation and cross-linked protein tyrosine [27], and it was proposed that activated neutrophils/tyrosine catalyzed LDL oxidation.

Phenolic antioxidants can be both pro-oxidative and antioxidative (Fig. 1), which suggests that dietary flavonoids/phenolics could be potentially more of an oxidative risk than a benefit [10]. We have shown that catalytic concentrations of flavonoids with a phenol B ring (e.g., apigenin, naringenin), upon oxidation by peroxidase/H_2O_2, formed phenoxyl radicals which catalyzed GSH or NADH co-oxidation and generated ROS [28–30]. The Trolox Equivalent Antioxidant Capacity TEAC assay method using 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) for ranking antioxidants could also be modified so that pro-oxidant activity could also be ranked [31]. Furthermore, we have also shown

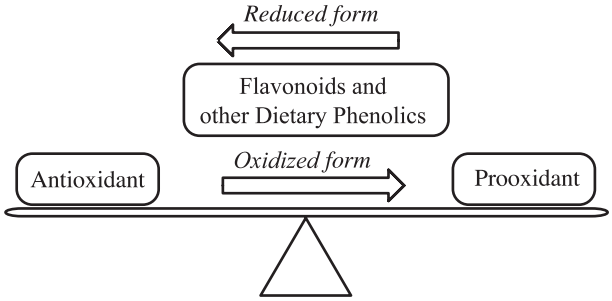


Fig. 1. Diagram representing the balance between antioxidant and pro-oxidant characteristics of flavonoids and other dietary phenolics. The reduced forms of flavonoids or other dietary phenolics act as antioxidants; however, the oxidized forms (phenoxyl radicals or quinone/quinone methide intermediates) can have pro-oxidant activities.

that phenol ring-containing dietary flavonoids and other phenolics also caused GSH oxidation in isolated rat hepatocytes [32]. Consumption of large amounts of flavonoids in the form of a concentrated supplement might not be considered safe until their in vivo potential for oxidative stress is evaluated.

In addition to our work regarding peroxidase-catalyzed oxidation of phenol B ring-containing flavonoids to pro-oxidant phenoxyl radicals, we and others have shown that peroxidase-mediated oxidation of catechol B ring-containing flavonoids results in the formation of semiquinone- and quinone-type metabolites. These semiquinone- and quinone-type metabolites may act as electrophiles binding to cellular macromolecules and may also result in the production of ROS through redox cycling [33]. Quercetin, the most ubiquitous of the dietary flavonoids, contains a catechol B ring and has been shown to be oxidized by tyrosinase, or hydrogen peroxide and horseradish peroxidase, or other peroxidases, to quinone/quinone methide intermediates, with subsequent reactions with GSH resulting in quercetin glutathionyl adducts [29,34,35]. Recently, quercetin has been shown to covalently bind to cellular DNA and protein in human intestinal Caco-2 cells and hepatic Hep G2 cells [36]. Thus, although it is well known that quercetin as an antioxidant flavonoid will affect the status of ROS in cells, several researchers have shown that the opposite occurs as well, specifically that ROS will metabolize certain flavonoids to species that covalently interact with key target macromolecules.

Pro-oxidant activity and its significance in cancer therapy

The beneficial effects of flavonoids in cancer therapy have often been linked to their ability to act as antioxidants, which includes their reducing capacities and ROS-scavenging capabilities. Interestingly, a recent review in this serial review series suggests that flavonoids

may not act as conventional hydrogen-donating antioxidants but may exert modulatory actions in cells through actions at protein kinase and lipid kinase signaling pathways [37]. The basis for this view is that flavonoids and their metabolites are not likely to act as major antioxidants in vivo because endogenous antioxidants such as ascorbic acid are at much higher concentrations. However, the flavonoid concentrations which do occur in vivo may be high enough to mediate receptor or enzyme activity. By inhibiting or stimulating various signaling pathways (e.g., tyrosine kinases, protein kinase C, and mitogen-activated protein kinase), flavonoids at low concentration could affect cellular function. Flavonoid phenoxyl radicals or semiquinone radicals described above may also inhibit cell proliferation signaling.

The chemopreventive properties of flavonoids are generally believed to reflect their ability to scavenge endogenous ROS. However, the pro-oxidant action of plant-derived phenolics rather than their antioxidant action may be an important mechanism for their anticancer and apoptosis-inducing properties, as ROS can mediate apoptotic DNA fragmentation [38–40]. The antioxidant properties of dietary phenolics may only partly explain their antitumor promotion effects, as ellagic acid is 10 times more potent an antioxidant than tannic acid. However, tannic acid was more effective than ellagic acid in inhibiting the promotion of skin tumor by 12-*O*-tetradecanoyl phorbol-13-acetate [41].

Apoptotic DNA fragmentation properties of several anticancer drugs are considered to be mediated by ROS [39]. Certain properties of dietary phenolic compounds, such as binding and cleavage of DNA and the generation of ROS in the presence of transition metal ions [40], are similar to those of known anticancer drugs. A putative mechanism for anticancer and apoptosis-inducing properties of plant-derived dietary phenolic compounds, which is a mechanism of DNA fragmentation that involves the mobilization of intracellular and extracellular copper, has been proposed [38]. As an example, the reaction mechanism of DNA cleavage by resveratrol and Cu^{2+} was investigated by another laboratory, which found that resveratrol forms a complex with Cu^{2+}, reducing it to Cu^{1+}, with the formation of one or more "oxidized species" of resveratrol [42]. The authors then demonstrated that the oxidized products of resveratrol are capable of reducing Cu^{2+} to Cu^{1+}. When calf thymus DNA was treated with increasing molar ratios of Cu^{2+} and resveratrol, the concomitant efficiency of DNA cleavage was increased [38].

Mitochondrial toxicity and its significance in cancer therapy

Another mechanism proposed for the anticancer and tumor cell apoptosis-inducing properties of flavonoids is that their pro-oxidant phenoxyl radicals cause mitochondrial toxicity by collapsing the mitochondrial membrane potential. Apoptosis (programmed cell death) is required to maintain a balance between cell proliferation and cell loss. Misregulation of this balance can lead to malignant transformation, whereas induction of apoptosis suppresses the development of cancer [43,44]. Various diet-derived compounds, e.g., resveratrol, have been shown to induce apoptosis in malignant cells and provide a promising natural strategy to prevent cancer [45,46]. Mitochondrial dysfunction contributes to a variety of human disorders, ranging from neurodegenerative and neuromuscular diseases to obesity, diabetes, ischemia–reperfusion injury, and cancer. Mitochondria, referred to as the "powerhouses" of the cell, have now also been recognized as the cell's "arsenal," reflecting their key role in apoptosis signaling. Increased pharmacological efforts have led to the emergence of mitochondrial medicine for manipulating mitochondrial functions so as to selectively protect, repair, or eradicate cells [47].

It has been proposed that opening the mitochondrial permeability transition pore (PTP) and/or mitochondrial membrane permeabilization (MMP) signals cell death by releasing apoptogenic factors, e.g., cytochrome *c*, from the mitochondria. Dissipation of the mitochondrial membrane potential ($\Delta\psi_m$) is an early event and the release of cytochrome *c* has been shown to occur in a variety of models relatively early during cell death [48–51]. Cells with collapsed $\Delta\psi_m$ are irreversibly committed to undergo death, even when the apoptosis-inducing trigger is withdrawn, and this marks the point of no return in the cell death process [52,53]. MMP is a function that cannot be lost simply because mammalian cells require mitochondria for survival [52]. Induction of MMP by drugs specifically designed to target mitochondrial receptors, either protein or lipid, has been shown to be efficient in the experimental treatment of cancers [54].

Curcumin, a dietary polyphenol, also opened the PTP and induced mitochondrial swelling, calcium release, and respiration impairment and collapsed the mitochondrial membrane potential [55,56]. Baicalin is a flavonoid and major component of the herbal medicine Sho-saiko-to, commonly used for treating chronic hepatitis in Japan and China. Baicalin induced apoptosis of Jurkat cells, a leukemia-derived T cell line, which was accompanied by intracellular ROS generation, mitochondrial cytochrome *c* release, and disruption of $\Delta\psi_m$ before activation of caspase 3. This suggests that baicalin acts as a pro-oxidant and induces mitochondrial-mediated apoptosis [57]. Nordihydroguaiaretic acid (NDGA) found in chaparral, an herbal medicine, is also a mitochondrial toxin [58]. Further-

more, the isoflavone analog rotenone is a classical complex I inhibitor of the mitochondrial respiratory chain [59] and its analogs have been shown to be effective anticancer agents [60].

Epidemiological studies suggest that consumption of green tea is associated with a lower risk of several types of cancers, including stomach, esophagus, and lung [61]. Administration of tea extract and green tea catechins reduced the growth of breast and prostate cancers in nude mice [62]. The growth suppressive effect of green tea flavonoids and gallic acids is at least partly due to the induction of apoptosis [63,64]. EGCG may be responsible for most of the anticancer activity of tea [65] and is currently the most effective agent for preventing colon cancer in rats [66,67]. Epigallocatechin and theaflavins have also been shown to have antiproliferative and anticarcinogenic activities [68–70]. Recently, mitochondrial depolarization and ROS formation were suggested to be early processes that lead to EGCG-induced apoptosis of human prostate and lung carcinoma cells [61,71]. Furthermore, cytotoxicity caused by propyl gallate (a food supplement and dietary phenolic) has been attributed to mitochondrial dysfunction [72].

Flavopiridol is widely used in traditional medicine and is a novel semisynthetic flavone analog of rohitukine, a leading anticancer compound derived from an Indian tree. Flavopiridol inhibits most cyclin-dependent kinases (CDKs) and is the first CDK inhibitor to be tested in human clinical trials by Aventis Pharma and the National Cancer Institute for the potential treatment of cancer [9]. Flavopiridol is effective against refractory cancers, in particular renal, prostate, and colon cancers, as well as lymphoma [73], and has achieved proof-of-concept in phase I/IIa trials [74]. Flavopiridol causes apoptosis in human chronic lymphocytic leukemia cells, and the cytotoxic mechanism involves caspase 3 activation [75,76]. However, it is not known if mitochondrial apoptosis is involved. The major dose-limiting flavopiridol side effect was severe diarrhea [77].

We have shown that cytotoxic flavonoids and other dietary phenolics were best at collapsing the hepatocyte mitochondrial membrane potential [7]. In addition, we have also shown that the dietary phenolic capsaicin inhibited Hep G2 cell proliferation and caused cytotoxicity in isolated rat hepatocytes likely by inducing mitochondrial toxicity. Capsaicin was shown to induce mitochondrial membrane potential collapse and cytotoxicity, which were prevented by mitochondrial membrane permeabilization transition pore inhibitors [78]. The flavonoids and other dietary phenolics that cause ROS formation in the presence of transition metals or peroxidases, and those which collapse mitochondrial membrane potential, are summarized in Table 1 and the flavonoid structures are shown in Table 2.

FLAVONOID EFFECTS ON DRUG-METABOLIZING ENZYMES AND ITS SIGNIFICANCE IN CHEMOPREVENTION

Preventing carcinogen metabolic activation by inhibiting drug-metabolizing enzymes

One important mechanism by which flavonoids may exert their in vivo chemopreventive effects is through their inhibition of phase I metabolizing enzymes, such as cytochrome P450 (CYP), which metabolically activates procarcinogens to reactive intermediates that trigger carcinogenesis [9,79,80]. Flavonoid interactions with CYPs have been reviewed recently [81]. Quercetin, kaempferol, and galangin are thought to be natural dietary ligands of the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor. Galangin in particular has been shown to inhibit AhR function and inhibit CYP1A1 thereby preventing the metabolism and activation of polycyclic aromatic hydrocarbons [80,81]. Resveratrol is also a selective inhibitor of human CYP1A1 (IC_{50} 11 μM) [82]. Large doses of ellagic acid were observed to prevent lung tumorigenesis induced by the tobacco carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, which was attributed to inhibition of lung cytochromes P450 [83]. Ellagic acid was also shown to inhibit CYP1A and CYP2E1 [84]. Capsaicin is responsible for the pungent and hot sensation of hot chili peppers and is widely used in spicy food. It is also a phenolic antioxidant with sensory neurotoxic effects and chemopreventive activity against chemical carcinogens and mutagens [85]. The chemoprotective action of capsaicin against tumorigenesis and mutagenesis by vinyl carbamate or *N*-nitrosodimethylamine was attributed to its inhibition of CYP2E1, which was responsible for activation of these carcinogens [86]. CYP1A and 2B were also inhibited and could explain the inhibition of benzo[*a*]pyrene-induced skin carcinogenesis by capsaicin [85,86].

A number of flavonoids, such as quercetin, fisetin, galangin, myricetin, kaempferol, chrysin, and apigenin, were also demonstrated to be potent inhibitors of the P-form phenol sulfotransferase (PST)-mediated sulfation (IC_{50} values < 1 mM) of acetaminophen and minoxidol by human liver cytosol [87]. The isoflavone genistein, and the other natural phenolic products curcumin and ellagic acid, were also inhibitors of P-form PST, with IC_{50} values of 0.38–34.8 mM. Quercetin was also shown to inhibit sulfoconjugation by Hep G2 cells. Although quercetin was less potent in this intact cell system, it was still more potent than 2,6-dichloro-4-nitrophenol, the classical P-form PST inhibitor, which has also been shown to inhibit

Table 1. Summary of Dietary Flavonoid/Phenolic Pro-oxidant Activity, Mitochondrial Toxicity, and Effects on Drug-Metabolizing Enzymes

Flavonoid or other dietary phenolic	Pro-oxidant activity or mitochondrial toxicity	Effects on cytochrome P450	Effects on phase II metabolizing enzymes	References
Flavonoid				
Quercetin	Pro-oxidant (transition metals)	Induces/inhibits CYP1A1	Inhibits PST, UGT; induces NQO	[14,80,81,91,145–147]
Galangin	Collapses $\Delta\psi_m$	Induces/inhibits CYP1A1	Inhibits PST, induces NQO	[7,87,145–147]
Diosmin		Induces CYP1A1		[144]
Diosmetin		Induces CYP1A1		[144]
Tangeretin		Induces CYP1A1/2		[81]
Flavone		Induces CYP1A1/2		[81]
β-Naphthoflavone		Induces CYP1A1/2		[81]
Kaempferol		Inhibits CYP1A1	Inhibits PST, induces NQO	[81,146,147]
α-Naphthoflavone		Inhibits CYP1A1/2, induces CYP3A4		[100]
Naringenin	Pro-oxidant (peroxidase)	Inhibits CYP3A4		[28,29,103,104]
Flavanone		Induces CYP2B1/2		[109]
Fisetin	Pro-oxidant (transition metals)		Inhibits PST, UGT	[14,87]
Myricetin	Pro-oxidant (transition metals)		Inhibits PST, induces NQO	[14,87]
Chrysin	Collapses $\Delta\psi_m$		Inhibits PST, UGT	[7,87,105]
Apigenin	Pro-oxidant (peroxidase)		Inhibits PST, UGT, induces NQO	[28,29,30,87,91,105]
4′-Bromoflavone			Induces NQO	[92]
Baicalin	Pro-oxidant Collapses $\Delta\psi_m$			[57]
Genistein			Inhibits PST	[87]
Other dietary phenolic				
Green/black tea phenolics	Collapses $\Delta\psi_m$		Induces GST, NQO, and UGT	[61,89,90]
Curcumin	Collapses $\Delta\psi_m$		Inhibits PST	[54,87]
Ellagic acid		Inhibits CYP1A1, CYP2E1	Inhibits PST	[83,84,87]
Hydroxycinnamic acids (phenol ring)	Pro-oxidant (peroxidase)			[32]
Hydroxycinnamic acids (catechol ring)	Pro-oxidant (transition metals)			[13]
Capsaicin	Pro-oxidant (peroxidase), collapses $\Delta\psi_m$	Inhibits CYP1A, CYP2B, CYP2E1		[7,32,79,85,86]
Resveratrol	Pro-oxidant (peroxidase and transition metals)	Inhibits CYP1A1		[32,42,82]

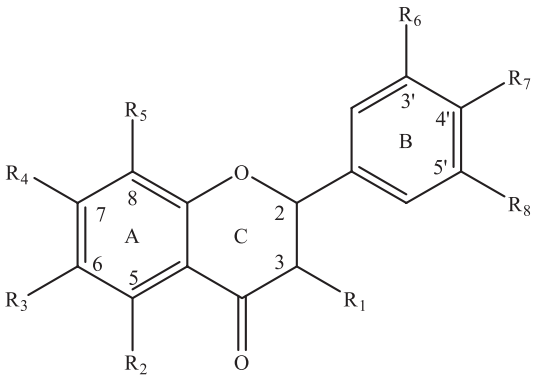
PST in vivo [87]. Sulfation is believed to activate carcinogens such as hydroxymethyl polycyclic aromatic hydrocarbons, allylic alcohols, benzylic alcohols, and *N*-hydroxyarylamines as their sulfate esters are electrophiles which covalently bind to nucleic acids and other macromolecules [88]. Flavonoids may therefore act as chemopreventive agents in sulfation-induced carcinogenesis.

Carcinogen metabolic detoxification by inducing phase II drug-metabolizing enzymes

Another mechanism claimed to be responsible for the chemopreventive activity of flavonoids is the induction of phase II metabolizing enzymes such as glutathione *S*-transferase (GST), NAD(P)H:quinone oxidoreductase (NQO), and UDP-glucuronyltransferase (UGT) [7], by which carcinogens are detoxified and therefore more readily eliminated from the body. Green and black tea extracts, which contain several flavonoids and other dietary phenolics, strongly inhibited neoplastic transformation in mammary organ cultures or epithelial cells, inhibited benzo[*a*]pyrene DNA adduct formation, and induced the phase II metabolizing enzymes GST, NQO [89], and UGT [90]. Phase II enzyme induction may explain the chemopreventive effect of tea in inhibiting heterocyclic amine-induced colonic aberrant crypt foci formation in the rat [67]. The order of effectiveness of flavonoids found for inducing NQO in murine hepatoma cells was galangin and kaempferol > quercetin > myricetin and apigenin, with epicatechin, catechin, and taxifolin being inactive [91]. The synthetic flavonoid 4′-bromoflavone was the most potent in vivo inducer of NQO and GSH synthesis enzymes and prevented mammary carcinogenesis in rats induced by polycyclic aromatic hydrocarbons [92].

The potent rat colon carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), unlike other

Table 2. Structural Formulas of Potentially Toxic Flavonoids



Flavonoid	Substituents								
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	C2-C3
Quercetin	OH	OH		OH		OH	OH		=
Galangin	OH	OH		OH					=
Diosmin		OH		O-Rut		OH	OCH_3		=
Diosmetin		OH		OH		OH	OCH_3		=
Tangeretin		OCH_3	OCH_3	OCH_3	OCH_3	H	OCH_3		=
Flavone									=
β-Naphthoflavone		Benzo	Benzo						=
Kaempferol	OH	OH		OH			OH		=
α-Naphthoflavone				Benzo	Benzo				=
Naringenin		OH		OH			OH		
Flavanone									
Fisetin	OH			OH		OH	OH		=
Myricetin	OH	OH		OH		OH	OH	OH	=
Chrysin		OH		OH					=
Apigenin		OH		OH			OH		=
4′-Bromoflavone							Br		=
Baicalin		OH	OH	O-Glc					=

Rut = 6-*O*-(6-Deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl; Glc = 5,6-dihydroxy-4-oxo-2-phenyl-4*H*-1-benzopyran-7-yl β-D-glucopyranosiduronic acid.

food-borne heterocyclic amines, did not induce tumors in rat liver. PhIP is efficiently detoxified in the liver and extremely low levels of PhIP–DNA adducts were formed in the liver. Depletion of hepatocyte GSH resulted in a 15-fold increase in the formation of PhIP–DNA adducts, as well as in a high level of unscheduled DNA synthesis [93]. Inhibition of hepatocyte glucuronidation with D-galactosamine (an inhibitor of glucuronidation) prevented *N*-hydroxy-PhIP glucuronidation and increased the formation of DNA adducts and unscheduled DNA synthesis 2-fold [93]. These results indicate that metabolic conjugation pathways involving GSH and glucuronidation may play an important role in protecting rat liver against PhIP carcinogenesis. Induction of UGT1A1 would also diminish the toxic actions of the anticancer drug irinotecan [94]. Dietary flavonoid/phenolic-mediated induction of UGT may be important for the glucuronidation and detoxification of colon and other carcinogens [90], as well as for the metabolism of therapeutic drugs [94].

Overcoming resistance to cancer therapy by inhibiting P-glycoprotein

Cancer therapy has been of limited success because of the intrinsic or acquired resistance of cancer cells to a broad range of chemically and functionally distinct anticancer agents, a phenomenon termed multidrug resistance (MDR) [95]. The classical form of MDR involves the overexpression of drug efflux transporters such as P-glycoprotein (P-gp) [96] and multidrug resistance-associated protein 1 [97], which reside in the cell membrane and pump anticancer drugs out of the cells, resulting in low intracellular drug concentrations. P-gp is responsible for the efflux of a broad array of hydrophobic compounds, including several important anticancer agents such as vinca alkaloids, anthracyclines, taxol, and epipodophyllotoxins [98]. The flavonoids biochanin

A, morin, phloretin, and silymarin were shown to inhibit P-gp-mediated drug efflux. Biochanin A and silymarin potentiated doxorubicin cytotoxicity in P-gp-positive cells [97]. The underlying mechanisms were proposed to involve direct interaction with P-gp as evidenced by flavonoid modulation of P-gp ATPase activity, inhibition of [^{3}H]azidopine photoaffinity labeling, and the observation that flavonoids did not change cellular P-gp levels [97]. It may be possible that some flavonoids may be used alone, or in combination with other P-gp inhibitors, to reverse MDR in the treatment of cancers.

SAFETY ISSUES: DIETARY FLAVONOID/PHENOLIC–DRUG INTERACTIONS AND ADVERSE EFFECTS

Flavonoid–drug interactions

Inhibition of CYPs, which are necessary for carcinogen activation, is a beneficial chemopreventive property of various flavonoids, but may be a potential toxic property in flavonoid–drug interactions. Inhibition of CYP activities by flavonoids has been extensively studied because of their potential use as agents blocking the initiation stage of carcinogenesis [99]. The general conclusion, upon analyzing the available data on CYP–flavonoid interactions, could be drawn that flavonoids possessing hydroxyl groups inhibit CYP activity, whereas those lacking hydroxyl groups may induce the enzyme [81]. Flavonoids can either inhibit or induce human CYPs depending upon their structures, concentrations, or experimental conditions, as α-naphthoflavone is an inhibitor of human CYP1A1 and 1A2 [100] but an inducer of CYP3A4 [101]. Interactions of flavonoids with CYP3A4, the predominant human hepatic and intestinal CYP responsible for metabolizing 50% of therapeutic agents as well as the activation of some carcinogens, is of particular interest [81]. The simultaneous administration of flavonoids and clinically used drugs may cause flavonoid–drug interactions by modulating the pharmacokinetics of certain drugs, which results in an increase in their toxicity or a decline in their therapeutic effect, depending on the flavonoid structure [102]. Naringenin (Fig. 2), a major flavanone present in grapefruit juice, exerts an inhibitory effect on intestinal CYP3A4 within 30 min and impairs the human metabolism of certain drugs such as those belonging to the class of calcium channel blockers (e.g., felodipine, nitrendipine, nisoldipine, verapamil) when co-administered with grapefruit juice [103]. Indiscriminate use of herbal products can also alter the pharmacokinetics of certain drugs and result in increased drug toxicity. This issue is particularly important in assessing the safety of concentrated flavonoid food supplements or herbal products particularly if their plasma concentrations stay high after ingestion [81]. Inhibition of CYPs by flavonoids may also be a concern when nontherapeutic agents are consumed. By adding polar groups to these xenobiotics, CYPs play a role in enhancing their elimination. Although beneficial as a chemopreventive property, inhibition of CYPs by flavonoids may inhibit the metabolism and elimination of these nondrug compounds, increase their accumulation in vivo, and cause toxicity.

Phase II metabolism is generally regarded as a detoxification pathway and inhibition of these enzymes can lead to increased toxicity of a xenobiotic. Indeed, inhibition of UGTs by flavonoids, when taken concomitant with a drug, can cause an overdose of the drug. Flavonoids have been shown to be substrates for UGTs and, therefore, when taken in combination with certain drugs, may inhibit their glucuronidation as a result of competitive inhibition. 2-[[4-[[2-(1*H*-Tetrazol-5-ylmethyl)phenyl]methoxy]phenoxyl]methyl] quinoline (RG 12525) is a new chemical entity recently evaluated for the treatment of type II diabetes, the tetrazole *N*2-glucuronide conjugate of which clinical studies have identified as the predominant metabolite in plasma after oral administration. A known substrate for glucuronidation is naringenin and it has been shown to inhibit 44% RG 12525 glucuronidation when used in equimolar concentrations [104]. Chrysin and apigenin, at concentrations that may be achieved in the diet, were both determined by HPLC and mass spectrometry to be glucuronidated and sulfated at the 7 position in human Caco-2 cells, Hep G2 cells, and isolated rat hepatocytes. No other metabolites were detected, suggesting that P450 did not play a role [105]. Table 1 summarizes the flavonoids and other dietary phenolics which modulate CYPs or phase II metabolizing enzymes. We have found that naringenin or quercetin (200 mg/kg) (which are glucuronidated) markedly increased the in vivo anesthetic duration and hepatotoxicity of propofol, a phenolic anesthetic, in mice as a result of inhibiting propofol glucuronidation (submitted for publication).

Flavonoid–carcinogen interactions

Induction of CYPs by flavonoids might also significantly affect the plasma concentrations of pharmaceutical drugs, resulting in a loss of therapeutic effect or an overdose. CYP induction could also increase the metabolic activation of carcinogens. Certain flavonoids like some other xenobiotics, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, induce CYPs via binding to AhR [106]. This mechanism is associated with an elevation in activities of the CYP1 family enzymes that are responsible for the activation of carcinogens such as meat-derived heterocyclic aromatic amines, benzo[*a*]pyrene, aflatoxin B_1, and 7,12-dimethylbenz[*a*]anthracene [107]. Many

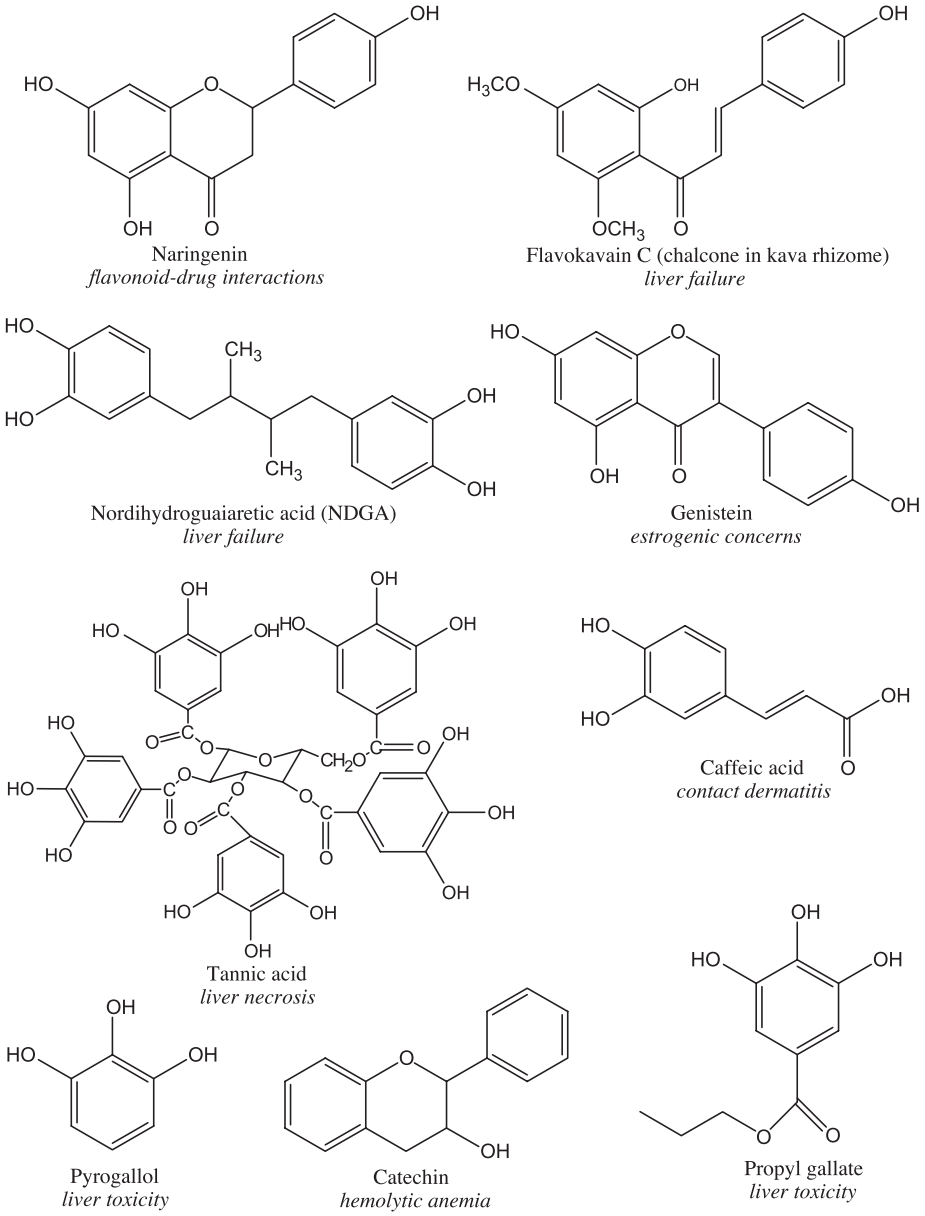


Fig. 2. Structures of toxic flavonoids and other dietary phenolics which may have need for concern in their use in therapy.

flavonoids are AhR ligands because the binding affinities for AhR seem to be largely dependent on structural constraints, including planar aromatic compounds with few bulky substituent groups [108]. Flavonoids have been shown to often act as AhR agonists and induce CYP1A1 and CYP1A2 activities. Galangin, quercetin, diosmin, and diosmetin are examples of flavonoids that increase transcription of the *CYP1A1* gene, whereas others, like flavone, tangeretin, and synthetic β-naphthoflavone, are flavonoids that induce CYP1A1/2 and to some extent CYP2B1/2 [81]. Flavanone is a specific inducer of CYP2B1/2 [109]. Aromatic amine carcinogens are metabolically activated by CYP1A2, whereas polycyclic aromatic hydrocarbon carcinogens are metabolically activated by CYP1A1.

Flavonoid and other dietary phenolic-induced systemic toxicity versus chemoprevention/chemotherapy observed in vivo

Epidermal growth factor receptor (EGFR)-associated protein tyrosine kinase (PTK) complexes have vital anti-apoptotic functions in human breast cancer cells [110]. It has been shown that targeting the naturally occurring PTK inhibitor genistein to the EGFR-associated PTK complexes using the EGF–genistein conjugate triggers rapid apoptotic cell death in human breast cancer cells in vitro.

An in vivo study in mice treated with EGF–genistein at dose levels as high as a single intraperitoneal (i.p.) 40 mg/kg dose, or 140 mg/kg administered i.p. over 28 consecutive days, did not cause any observed toxicities. EGF–genistein significantly improved tumor-free survival in a severe combined immune deficiency mouse xenograft model of human breast cancer when it was administered 24 h after inoculation of tumor cells. Interestingly, at 100 μg/kg/d for 10 d (i.e., 1 mg/kg total dose, which is >100 fold less than the highest tested and nontoxic cumulative dose) in mice, EGF–genistein was more effective than cyclophosphamide (50 mg/kg/d for 2 d), adriamycin (2.5 mg/kg for 1 d), or methotrexate (0.5 mg/kg for 1 d), the most widely used standard anticancer drugs for breast cancer, and resulted in 60% long-term tumor-free survival [110]. This study reveals that an isoflavone may show anticancer properties at a much lower dose than the dose which causes systemic toxicity.

The isoflavone daidzein was recently studied in female rats for its potential to alter fertility and for chemoprevention to the mammary gland [111]. Doses of 250 mg/kg and 1 g/kg fed to female rats 2 weeks before breeding and continued until the offspring were 50 d postpartum did not have a significant effect on fertility or numbers of male and female offspring. However, the higher dose did result in reduced progesterone levels, slight decreases in ovarian and uterine weights and mammary gland size, and reduced body weight, a fact that may be explained by reduced feed consumption. Perinatal exposure of female offspring to 250 mg/kg daidzein did not alter mammary gland development or ontogeny of chemically induced mammary tumors in rats treated with dimethylbenz[*a*]anthracene (DMBA) on day 50. The authors concluded that supraphysiological concentrations of daidzein administered via the diet did not cause significant toxicity to the female reproductive tract or provide a protective effect against chemically induced mammary cancer [111]. Interestingly, in a separate study conducted by a separate laboratory, phenoxodiol (a novel isoflavone derivative) was shown to inhibit DMBA-induced mammary carcinogenesis in female rats [112]. Tumor multiplicity was reduced by 42% in rats fed 50 mg/kg phenoxodiol and by 49% in rats fed 75 mg/kg phenoxodiol. The authors concluded that phenoxodiol is an effective chemopreventive agent against DMBA-induced mammary carcinogenesis. Systemic toxicity with phenoxodiol was not evaluated in this study.

In contrast to the above study using daidzein, which showed little female reproductive toxicity at a high dose, a recent study reported significant male reproductive toxicity when male rats were treated with flavonoid-rich seed extracts of *Vitex negundo* (commonly called nirgundi) [113]. Four flavonoids, namely acerosin, trimethoxywogonic, artemin, and isopyrenin, have been identified from this extract. All the major accessory sex organs shed weight when the preparation was administered at doses of 15 mg/rat/d after 15 d of treatment. This is a rather low dose. The drop in weight was also reflected in disturbed tissue biochemistry (e.g., secretory products diminished). Factors that may impede fertility, such as a reduction in sperm numbers and slackness in their motility, were observed. Toxicity testing in blood did not point to distress in any of the vital organs. It was concluded that the seed extracts of *V. negundo* did interfere with male reproductive function, although no adverse toxicity was observed in other vital organs [113].

The phenol B ring-containing flavonoid naringenin, which was shown to have pro-oxidant activity and inhibit CYP3A4, was also shown to have lethal and teratogenic effects as evaluated by means of an amphibian embryo toxicity test. Amphibians are being increasingly used for toxicity screening purposes due to their high sensitivity to physicochemical stress [114]. Doses of 10 mg/l naringenin exerted 100% malformations and caused 30% of the abnormal embryos to die. Main abnormalities were reduced body size, axial curves, micronephaly, underdeveloped gills, abdominal edema, and delayed development. Naringenin and several other polyphenolic compounds were tested for their inhibition of lung metastasis induced by B16F10 melanoma cells in mice [115]. Oral administration of naringenin at a concentration of 200 nmol/kg body weight was found to inhibit 26% of lung metastasis, as seen by the reduction in the number of lung tumor nodules.

Pyrogallol (Fig. 2), a simple phenolic found in green tea, was shown to cause significant hepatic damage in rats when administered i.p. at 100 mg/kg. The serum enzymes aspartate aminotransferase and alanine aminotransferase (ALT) were increased, as well as malondialdehyde [116], suggesting that free radical formation and pro-oxidant toxicity played a role. The propyl, octyl, and dodecyl esters of gallic acid have been studied extensively in a significant number of animal experiments involving oral dosing. Knowledge of the toxicity of pyrogallol and gallates is important when evaluating flavonoid toxicity, as several major tea catechins, such as EGCG, contain pyrogallol and/or gallate moieties. In toxicity studies with propyl gallate (Fig. 2), growth retardation, kidney or liver changes, hyperplasia of the forestomach, and anemia were the most prominent effects at dose levels above 10,000 mg/kg feed, and liver enzyme induction was seen at 5000 mg/kg feed [117]. The toxicity of pyrogallol and gallate is particularly interesting as these may contribute to EGCG (a tea catechin) toxicity, as EGCG has both a pyrogallol and a gallic acid moiety. Our laboratory has also investigated the toxicity of propyl

gallate, gallic acid, and EGCG in vivo in mice and found that all three caused significant increases in plasma ALT levels characteristic of liver injury. It was found that EGCG (120 mg/kg), propyl gallate (170 mg/kg), and gallic acid (500 mg/kg) caused a 4-fold increase in plasma ALT levels after 24 h when injected i.p. into CD-1 mice [118]. These results suggest that tea flavonoids and other phenolics caused significant hepatic damage in vivo in rodent models. As a comparison of systemic toxicity with chemoprevention, a study of the inhibitory effects of EGCG on *N*-nitrosomethylbenzylamine-induced esophageal tumorigenesis in F344 rats is described here. In rats treated with EGCG at doses of 4 mg/kg i.p., or 10 mg/kg both orally and i.p., the mean number of tumors per rat was significantly reduced to 48, 56, and 61%, respectively [119].

Tannins (commonly referred to as tannic acid) are defined as water-soluble polymeric phenolics that precipitate proteins [120]. Proanthocyanidin is a condensed tannin and is a polymeric flavanol that accumulates in a number of tissues in a wide variety of plants. Tannic acid (Fig. 2) is present in many plant foods and herbal medicine and may exert various biological effects such as inhibiting H^+,K^+-ATPases [121], antioxidant enzymes [122], and drug-metabolizing enzymes [123]. Many animal studies suggest that tea tannic acid and other tea polyphenols are anticarcinogenic. Intraperitoneal injections of 10 mg/kg tannic acid into hairless mice afforded protection against UVB-induced papillomas [124]. Dietary feeding of proanthocyanidins extracted from grape seeds (0.2 and 0.5%, w/w) in AIN76 control diet to SKH-1 hairless mice resulted in prevention of photocarcinogenesis in terms of tumor incidence (20–95%), tumor multiplicity (46–95%), and tumor size (29–94%) against UVB-induced complete and initiation and promotion stages of photocarcinogenesis [125]. However, tannic acid has also been reported to induce liver necrosis [126], which was suggested to be partially due to the formation of pro-oxidant intermediates and inhibition of antioxidant enzymes, as tannic acid induced oxidative stress in fish liver after an i.p. dose of 10 mg/kg [122]. We observed that tannic acid (120 mg/kg i.p.), when administered to CD-1 mice, caused a 4-fold increase in plasma ALT levels after 24 h [118], also suggesting liver injury. Table 3 provides a summary of the specific in vivo studies described above, listing the doses of several flavonoids and other dietary phenolics described here which were required to cause systemic toxicity or prevent carcinogenesis in various animal models.

Adverse effects of flavonoids and other dietary phenolics observed in humans

Cyanidanol is the drug name for the flavonoid catechin (Fig. 2). One study reports that three patients administered cyanidanol developed both hemolytic anemia and thrombocytopenia, whereas two had only thrombocytopenia. Upon suspension of the drug, the hematological values returned to normal [127]. Drug-dependent platelet antibodies were detected in four of the five patients and three patients had cyanidanol-dependent red blood cell antibodies.

Kava (*Piper methysticum*) is a shrub indigenous to the islands of the South Pacific [128], and its constituents include lactone derivatives known as kavalactones and three kavachalcones, such as flavokavain C (Fig. 2) [129]. Chalcones are precursors to flavonoids which differ by the absence of a closed C ring. Kava is used traditionally in the South Pacific and has more recently been marketed in the West as an anxiolytic and mood enhancer [130]. Case reports detailing liver failure after ingestion of kava have been emerging over the past few years, and over-the-counter sales of these herbal preparations have recently been banned in Switzerland and

Table 3. Summary of Doses which Were Required for Systemic Toxicity or Chemoprevention/Chemotherapy in Single in Vivo Studies

Flavonoid or other dietary phenolic	Systemic toxicity dose	Chemoprevention/chemotherapy dose	References
Isoflavone			
Genistein	140 mg/kg (mice) (no effect)	1 mg/kg (mice) (60% long-term tumor-free survival)	[110]
Daidzein	250 mg/kg (rats) (no effect)	250 mg/kg (rats) (no effect)	[111]
Phenoxodiol		50 mg/kg (rats) (tumor multiplicity reduced by 42%)	[112]
Other flavonoid			
Flavonoids of *Vitex negundo*	15 mg/day (rats) (male reproductive toxicity)		[113]
Naringenin	10 mg/l (amphibian) (teratogenic)	200 nmol/kg oral (mice) (inhibited 26% of lung tumor nodule formation)	[114,115]
EGCG	100 mg/kg i.p. (mice) (hepatotoxicity)	4 mg/kg i.p. (rats) (mean number of tumors decreased)	[118,119]
Other phenolic			
Pyrogallol	100 mg/kg i.p. (rats) (hepatotoxicity)		[116]
Propyl gallate	5 g/kg oral (rats) (hepatotoxicity)		[117]
Tannic acid	10 mg/kg i.p. (fish, carp); 120 mg/kg i.p. (mice) (hepatotoxicity)	10 mg/kg i.p. (mice) (protection against papillomas)	[118,122,124]

Germany, while Britain is also proposing a ban [131]. Recently, more than 30 cases of kava-associated hepatic injury have been described, including 5 cases leading to orthotopic liver transplantation. Whole kava extracts and the kavalactones bearing a methylenedioxyphenyl moiety caused concentration-dependent decreases in CYP2C9 and CYP2C19 > CYP3A4 and CYP2D6 > CYP4A9/11 and CYP1A2 [132]. Kavalactones are pyrones which form 96% of the kava lipidic extracts and are metabolized to phenols. These CYP effects indicate that kava has a high potential for causing drug interactions through inhibition of CYP enzymes responsible for the majority of the metabolism of pharmaceutical agents. Kava may augment the effects of the benzodiazepine alprazolam, resulting in a coma, as well as ketoconazole [132].

NDGA is a constituent of the creosote bush *Larrea divaricata* and is a lignan found in high amounts (up to 10% by dry weight) in the leaves and twigs of *Larrea tridentate*, which is an ethnobotanically important plant found in the American Southwest and northern Mexico. NDGA (Fig. 2) is the major lignan in chaparral. Chaparral has been used for thousands of years by Native Americans for a variety of purposes. It has been employed primarily in tea form to help with cramping pains, joint pains, and allergic problems, as well as to eliminate parasites. Externally it has been applied to reduce inflammation and pain and to promote healing of minor wounds. Chaparral takes its name from the area in which it grows, the desert regions of the southwestern United States and northern Mexico known as the chaparral ecosystem. The leaves and stems of this ancient plant are used as medicine. Although numerous beneficial effects have been attributed to this plant, several case reports have demonstrated that high doses of *Larrea*-containing herbals induce hepatotoxicity and nephrotoxicity in humans. NDGA has been shown to induce cystic nephropathy in the rat and it has also been reported that intraperitoneal administration of NDGA is lethal in the mouse (LD_{50}, 75 mg/kg) [133]. Administration is associated with a time- and dose-dependent increase in serum alanine aminotransferase levels, which suggests liver damage. We observed a 4-fold increase in plasma ALT levels after 24 h when NDGA at a dose of 50 mg/kg was administered i.p. to CD-1 mice [118].

Propolis ("bee glue") is produced by bees during hive construction to fill structural gaps. Allergic contact dermatitis induced by propolis has been attributed to its content of a catechol ring containing caffeic acid (Fig. 2) derivative, 1,1-dimethylallyl caffeic acid [134]. Since ancient times, propolis has been incorporated into numerous medical and cosmetic products as it is believed to be a potent antiseptic, anti-inflammatory agent, astringent, antioxidant, and local anesthetic. It can be found in toothpaste, mouthwash preparations, facial creams, ointments, chewing gum, polishes, and varnishes [134]. Clinical allergy in humans presents as an allergic contact dermatitis or oral mucositis, with the main population affected being beekeepers. This has recently increased among biocosmetic users because of the increasing popularity of propolis-containing "natural-based products" [135]. Recently, a case of a stringed instrument craftsman who developed allergic contact dermatitis to propolis, a component of Italian varnish, was reported [135].

Phytoestrogens and possible human toxicity

It is now realized that certain compounds, used in a wide variety of products, can mimic the effects of the main natural estrogen, 17β-estradiol, by binding to the estrogen receptor and influencing the expression of estrogen-dependent genes. Epidemiological data suggest a progressive decline in human male reproductive health and fertility [136] which may be associated with exposure to endocrine-disrupting chemicals in the environment and, more specifically, those which mimic the action of natural estrogens [137]. The importance of estrogen action on male reproduction is illustrated by the fact that the α and β forms of the estrogen receptor are expressed alone or together throughout the male reproductive tract [138].

Phytoestrogens are phenolic nonsteroidal plant compounds with estrogen-like biological activity. Most flavonoids are nonestrogenic or weakly estrogenic; however, the isoflavones such as genistein, other flavonoids such as apigenin and kaempferol, and the polyphenolic stilbenes such as resveratrol act through estrogen receptor-mediated mechanisms and also have antiestrogenic effects [139]. The use of phytoestrogens to protect against hormone-dependent cancers or as a "natural" alternative to hormone replacement therapy remains controversial. There is a paucity of data on endocrine effects of soy phytochemicals, such as genistein and daidzein, during infancy, the most sensitive period of life for the induction of toxicity. The safety of isoflavones in infant formulas has been questioned recently owing to reports of possible hormonal effects [140]. Closer studies in experimental animals and human populations exposed to phytoestrogen-containing products, and particularly soy-based infant formulas, are necessary as the estrogenic activity of genistein has been shown to be associated with decreased fertility and increased sexual dysfunction in experimental animals at high doses [141]. There is also an increased risk for breast and reproductive tract cancers in humans. Estrogenic activity in soybean-related foodstuffs is mostly due to genistein, whereas 8-prenylnaringenin is the active principle in beer and is 10 times more active than genistein [142].

CONCLUSIONS

Flavonoids, including isoflavones, are present in the diet and in a variety of dietary supplements, as are other similar plant phenolics with similar structures and pharmacological properties. The animal research studies into the beneficial effects of flavonoids in chemoprevention, as well as the tissue culture studies on the flavonoid-inducing cancer cell apoptosis effects, have been reviewed. With the increasing interest in alternative medicine, herbal products are ingested by at least 10% of the general population and 30–70% of individuals with specific disease states [143]. Because dietary supplements are not classified as drugs and do not require FDA approval to be marketed, potential toxicities and drug interactions are not evaluated thoroughly.

It is clear from this review that potential safety issues exist if megadoses of flavonoids and isoflavones were consumed daily. Phenol ring-containing flavonoids, upon oxidation by peroxidases, yield phenoxyl radicals which are cytotoxic; co-oxidize unsaturated lipids, GSH, NADH, ascorbate, and nucleic acids; and cause ROS formation and mitochondrial toxicity [7,28–30]. Catechol ring-containing flavonoids have been shown to form electrophilic quinone/quinone methide intermediates which bind to DNA, protein, and GSH [29,33–36]. Hepatotoxicity in mice was induced by the flavonoid epigallocatechin gallate (found in green tea) and the phenolics propyl gallate (a food supplement) and NDGA (found in a herbal medicine) when administered i.p. to mice [118]. Case histories of hepatotoxicity induced by NDGA or kava extracts have also been published. Additional reasons for concern about mega flavonoid supplements include potential flavonoid–drug interactions, as flavonoids have been shown to both induce [144] and inhibit [145] drug-metabolizing enzymes. It is evident that further research regarding the potential toxicities associated with flavonoids and other dietary phenolics is required if these plant-derived products are to be used as therapy.

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ABBREVIATIONS

AhR — aryl hydrocarbon receptor
ALT — alanine aminotransferase
CDK — cyclin-dependent kinase
CYP — cytochrome P450
$\Delta\psi_m$ — mitochondrial membrane potential
DMBA — dimethylbenz[*a*]anthracene
EGCG — epigallocatechin gallate
EGFR — epidermal growth factor receptor
GSH — glutathione
GST — glutathione *S*-transferase
HPLC — high-performance liquid chromatography
LDL — low density protein
MDR — multidrug resistance
MMP — mitochondrial membrane permeabilization
NDGA — nordihydroguaiaretic acid
NQO — NAD(P)H:quinine oxidoreductase
P-gp — P-glycoprotein
PhIP — 2-amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine
PST — phenol sulfotransferase
PTK — protein tyrosine kinase
PTP — permeability transition pore
RG 12525 — 2-[[4-[[2-(1*H*-tetrazol-5-ylmethyl)phenyl]-methoxy]phenoxyl]methyl]quinoline
ROS — reactive oxygen species
UGT — uridine diphosphate glucuronyltransferase